IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| APPLICANT(s): | Monica Petronella<br>Maria De Maat | | |
| SERIAL NO.: | 10/511,700 | ART UNIT: | 1651 |
| FILING DATE: | 10/15/2004 | EXAMINER: | Saucier, Sandra E |
| TITLE: | MODIFICATION OF THE PROPERTIES OF A FIBRIN MATRIX WITH RESPECT TO GROWTH AND INGROWTH OF CELLS | | |
| ATTORNEY DOCKET NO.: | 100137-56 | | |

CUSTOMER NO.: 021001

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**DECLARATION OF PIETER KOOLWIJK, PhD**

I, the undersigned Pieter Koolwijk, do hereby declare and state that:

1. I am a citizen of the Netherlands currently residing at Langewald 23, 1831 BL, Koedijk, The Netherlands.

2. I am a cell biologist currently working as scientist in the field of angiogenesis, specialized on matrices for accelerating and decelerating angiogenesis and the influence of hypoxia on angiogenesis at VU University Medical Center, Dept. for Physiology, Van der Boechorststraat 7, 1081 BT

Amsterdam, The Netherlands, formerly working at TNO Quality of Life, business unit Biosciences, assignee of the subject patent application, where I held the position of research scientist.

3. I am an expert in the field of the invention described and claimed in the subject patent application, as shown by my work (mentioned above) as a cell biologist in the field of angiogenesis, specialized on matrices for accelerating and decelerating angiogenesis and the influence of hypoxia on angiogenesis and, as shown by my *curriculum vitae*, attached hereto, and authorship and co-authorship of numerous scientific publications relating to angiogenesis, a list of which publications is provided in the attached Appendix.

4. I am familiar with the objections raised in the Office Communication of the USPTO of September 28, 2009, in patent application no. 10/511,700 and the question of enablement of the invention *in vivo*, i.e., the effect of the composition comprising fibrinogen and a pharmaceutically acceptable carrier on accelerating or decelerating of angiogenesis *in vivo*.

5. With respect to the aforementioned Office Communication, I have considered the disclosure of the captioned application for patent in light of the disclosure of Kaijzel et al., Journal of Thrombosis and Haemostasis, 4, 1975-1981, 2006, made of record by the examiner, with regard to the *in vivo* effect of the presently claimed method.

6. Kaijzel et al. disclose *in vitro* and *in vivo* studies to

investigate the influence of fibrinogen heterogeneity on angiogenesis in *in vitro* and in vivo angiogenesis models. Cell culture described under materials and methods of Kaijzel et al. (see p. 1976, right col., 2nd paragraph), used in these studies, corresponds to the disclosure of [0059] of the present application. In both cases, human foreskin microvascular endothelial cells (hMVEC) were cultured until confluence at 5 % $CO_2$ / 95% air on fibronectin-coated dishes in M199 supplemented with L-glutamine, HEPES, heat-inactivated human and calf serum, endothelial cell growth factor supplement, heparin, penicillin, and streptomycin. Confluent endothelial cells were used at passage 10 for *in vitro* angiogenesis.

7. The *in vitro* angiogenesis test described by Kaijzel et al. (see p. 1976, right col., 3rd paragraph) corresponds to the test disclosed in [0083] and [0085] of the present application. In both documents is described that the endothelial cells were detached from the fibronectin-coated or gelatin-coated culture plates by means of trypsin/EDTA and directly confluently seeded on three-dimensional fibrin matrices, optionally comprising factor XIIIa and $CaCl_2$. After 24 hour and subsequently 48 hours the cells were stimulated with M199, human and calf serum, bFGF, and TNFa.

8. Therefore, the *in vitro* data and results shown in Kaijzel et al. (see p. 1977, left col., last paragraph to p. 1978, left col., first paragraph) correspond to the *in vitro* data and results presented in the present application (see [0086] to [0090]). The *in vitro* assays disclose that the heterogeneity in naturally occurring fibrinogen influences the ingrowth of

blood vessels in the fibrin matrix. Thus, the HMVEC show an accelerated ingrowth in a fibrin matrix formed from the HMW form of fibrinogen relative to the total (unfractionated, mixed) fibrinogen. When the vessel ingrowth in fibrin matrices formed from the LMW form of fibrinogen was considered, even after 10 days of stimulation no vessel-like structure was formed. In HMW and LMW mixtures, it was clear that the greater the percentage of LMW fibrinogen, the less fast the angiogenesis took place.

9. The present application is not limited to *in vitro* processes, but also refers to *in vivo* processes (see specification, p. 3-4, bridging [0036]), even if the application does not provide explicitly *in vivo* data. Such data are described in Kaijzel et al., which further support and prove the *in vitro* effects.

10. The *in vivo* angiogenesis test were performed with FVB/N-TIE2/GFP mice, in which the vascular endothelial cells express green fluorescent protein (GFP) under the direction of the endothelial-specific receptor kinase (Tie2) promoter, and wildtype FVB/N mice as control animals. The experiments were repeated in a second mouse model, which was the *in vivo* model for angiogenesis of Kragh et al. (see Kaijzel et al., p. 1976, right col., last paragraph to p. 1977, left col., first paragraph). The results gained with the *in vivo* model confirm the above mentioned results of the *in vitro* angiogenesis model (see Kaijzel et al., p. 1978, left col., last paragraph to p. 1979, left col., first paragraph), and thus, confirm the disclosure of the present application.

11. It is a fact that angiogenesis is an essential process

during embryonic development, in the female reproductive system and in wound healing, but is also associated with pathological conditions such as chronic inflammations, rheumatoid arthritis, tumors and retinopathy in diabetics (see specification, p. 1, [0012]. Depending on the positive or negative effect of angiogenesis it is the aim to increase or decrease angiogenesis. The subject matter of the present application teaches methods allowing accelerating or decelerating angiogenesis by administering a fibrin matrix comprising at least 80 % HMW form of fibrinogen or at least 40 % LMW form of fibrinogen based on the results of the *in vitro* and *in vivo* angiogenesis models.

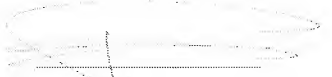
12. In case of external defects such as burns, tumors or inflammation the fibrin matrix is topically administered, whereas in case of internal defects such as tumors or chronic inflammations the fibrin matrix is also administered via intravenous injection or infusion (see specification, p. 4, [0040]).

13. Hence, the subject matter of the present application provides all the information to enable a person skilled in the art to rework the invention regarding methods for *in vitro* and *in vivo* modification of angiogenesis.

14. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Further declarant sayeth naught.



Pieter Koolwijk

25 January 2010

Date